Proc. Natl. Acad. Sci. USA
Vol. 76, No. 10, pp. 5377–5381, October 1979
Neurobiology

β-Endorphin: Analgesic and hormonal effects in humans

(metabolic clearance rate/prolactin/growth hormone/enkephalin analog/baboons)

KATHLEEN M. FOLEY*, IONE A. KOURIDES*, CHARLES E. INTURRISI*, ROBERT F. KAIKO*, CHARLES G. ZAROULIS*, JEROME B. POSNER*, RAYMOND W. HOUDE*, AND CHOH HAO LI†

*Memorial Sloan-Kettering Cancer Center and Cornell University Medical College, New York, New York 10021; and †Hormone Research Laboratory, University of California Medical Center, San Francisco, California 94143

Contributed by Choh Hao Li, June 18, 1979

ABSTRACT **The pharmacokinetics and the hormonal, analgesic, and behavioral effects of several doses of human β-endorphin were evaluated after intravenous administration to three patients and intracerebroventricular administration to one patient with pain caused by cancer. These effects were compared to the hormonal effects of intravenously administered morphine sulfate in two patients and an enkephalin analog in two baboons. The mean terminal half-life after intravenous administration of 5 or 10 mg of human β-endorphin to three patients was 37 min; the mean volume of distribution was 178 ml/kg, and the metabolic clearance rate was 3.2 (ml/min)/kg. The half-life of β-endorphin in cerebrospinal fluid after intracerebroventricular administration was 93 min, and the volume of distribution was 0.74 ml/kg. A rapid rise in plasma prolactin followed both intravenous and intracerebroventricular β-endorphin. Intravenous administration did not affect plasma growth hormone, but intracerebroventricular administration suppressed plasma growth hormone. No significant change in plasma growth hormone was noted after intravenous administration of morphine to humans, but plasma growth hormone decreased in one baboon after administration of the enkephalin analog. β-Endorphin-stimulated release of prolactin occurred at doses lower than those required to produce analgesic and other behavioral effects. When both hormonal and analgesic effects were observed (after 7.5 mg were given intracerebroventricularly), the onset of the hormonal response slightly preceded the analgesic and behavioral responses. These studies suggest that the hormonal effects of β-endorphin are species dependent and are similar to those of morphine. Hormonal and analgesic effects of β-endorphin appear to result from the activation of opiate receptors that differ in their locations and characteristics.**

Intracerebroventricular (icv) administration of β-endorphin causes similar physiological and pharmacological effects in several animal species (1, 2). In contrast, intravenous (iv) administration of β-endorphin causes different effects in different species. In the rat there is no demonstrable behavioral effect after iv doses of β-endorphin up to 30 mg/kg (3), whereas in the cat doses as low as 50–500 μg/kg produce behavioral changes (4). Preliminary studies in humans (5–7), using a wide dose range of human β-endorphin (β_h-ep), revealed both behavioral and analgesic effects. In the present study, we have assessed the pharmacologic disposition, as well as the concurrent hormonal, analgesic, and behavioral effects of β_h-ep administered in various doses iv to three cancer patients with pain and icv to one other cancer patient with pain. We administered an enkephalin analog, [2-D-alanine,5-norleucine]enkephalinamide, to two baboons to evaluate whether the hormonal and behavioral effects of opioid peptides were similar in nonhuman primates without pain or previous exposure to opiate drugs.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Experimental Subjects. Three patients with pain caused by cancer but no recent narcotic exposure received β_h-ep iv: Patient 1, a 65-year-old man with Hodgkin disease, received 1 mg and 5 mg on separate occasions. Patient 2, a 62-year-old woman with breast cancer, received 10 mg of β_h-ep and 10 mg of morphine sulfate iv on separate occasions. Patient 3, a 45-year-old man with diffuse histiocytic lymphoma, received 10 mg of β_h-ep. An additional patient, patient 4, received the drug by injection into the cerebral ventricle. He was a 43-year-old man with carcinoma of the lung, leptomeningeal metastases, and intractable pain. He had been taking oxycodone orally at 60 mg daily for 4 weeks prior to the study. β_h-ep was administered directly into a subcutaneous reservoir attached to a catheter located in the right lateral ventricle of the brain, in doses of 100 μg to 7.5 mg at 1–2 week intervals over a 10-week period; the reservoir had been inserted to treat the leptomeningeal tumor (8). Patient 3 also had a ventricular catheter permitting collection of cerebrospinal fluid (CSF) after iv β_h-ep administration. Two other patients with pain, patients 5 and 6, received 10 mg of morphine sulfate iv for comparative studies. Patient 5 was a 35-year-old man with chronic pain who had been taking meperidine orally at 600 mg daily for 20 weeks; patient 6 was a 52-year-old man with chronic lymphocytic leukemia and mild pain who had not been taking any analgesic drugs. Each patient was informed about the experimental nature of the study and agreed in writing to participate.

Two healthy 25-kg male baboons received an enkephalin analog, [2-D-alanine,5-norleucine]enkephalinamide iv in a dose of 2 or 4 mg. The baboons received no other medication except the nonnarcotic tranquilizer phencyclidine hydrochloride, in a dose of 25 mg intramuscularly, 30 min prior to the administration of the enkephalin analog.

Study Design. Test medications were administered under single-blind conditions. Subjective and objective measurements were assessed, and venous blood and CSF samples were collected by nurse-observers from each human subject prior to and at selected intervals for up to 24 hr after administration. Vital signs were measured at 5 to 10-min intervals. Changes in pain intensity and mood were assessed by using visual analog scales (VAS). The pain intensity VAS was a 10-cm line labeled "least possible pain" on the extreme left and "worst possible pain" on the extreme right. Patients marked in pencil the place on the line that best reflected their pain intensity. The mood VAS was similar except that the line was labeled "worst I could feel" on the left and "best I could feel" on the right. The pain intensity and mood VAS scores were determined by measuring the dis-

Abbreviations: β_h-ep, human β-endorphin; iv, intravenous(ly); CSF, cerebrospinal fluid; icv, intracerebroventricular(ly); RIA, radioimmunoassay; VAS, visual analog scales.

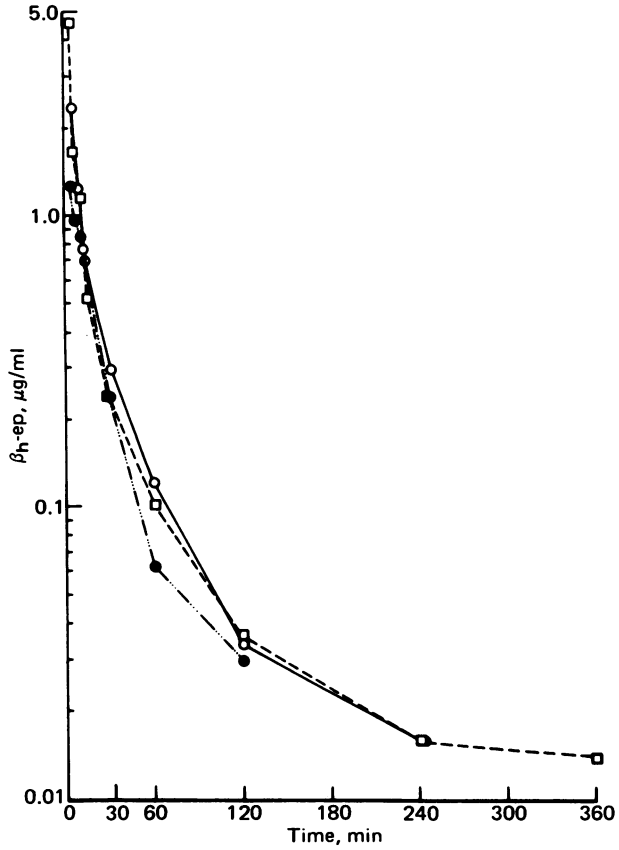

FIG. 1. Plasma concentrations of immunoreactive β_h-ep after iv administration of 5 (●) or 10 (○, □) mg of β_h-ep to three patients with pain.

tance in cm from the extreme left of the line to the patient's mark. These scales are modifications of the tests described and validated by Folstein and Luria (9). Pupil size was determined photographically at 15-min intervals throughout the study (10). In the baboon, vital signs were measured frequently, and venous blood samples were collected at selected intervals for up to 2 hr after medication administration. Blood and CSF samples were immediately placed at 4°C. Plasma was rapidly separated from blood cells. All samples were stored at −20°C until hormonal assays were performed.

Radioimmunoassays. β_h-ep was measured in biologic fluids by using a double antibody radioimmunoassay (RIA) developed by Kourides and coworkers (unpublished). This assay can reliably detect 50 pg/ml in the assay tube in unextracted plasma or CSF. Human β-lipotropin demonstrated a 10% (wt/wt) crossreactivity in the β_h-ep RIA; [leucine]- and [methionine]-enkephalin showed no significant crossreactivity. Gel chromatography of plasma from patient 2, 30 min after injection of β_h-ep iv, showed that all immunoreactive material, as measured in the β_h-ep RIA, coeluted with ^{125}I-labeled β_h-ep. Morphine was also measured by RIA, using a minor modification of the method of Spector (11). Prolactin, growth hormone, and thyrotropin were also measured by previously published RIA methods (12, 13). Normal human basal values in these assays are the following: prolactin, <2–12 ng/ml; growth hormone, <5 ng/ml; thyrotropin, 0.5–3.2 μU/ml.

Table 1. Pharmacokinetic parameters for β_h-ep and morphine

Patient	Drug	Dose mg	$t_{1/2}\alpha$, min^{-1}	$t_{1/2}\beta$, min^{-1}	Cl, (ml/min)/kg	V_d, ml/kg
1	β_h-ep	5	7.4	27	2.1	83
2	β_h-ep	10	6.7	39	4.8	268
3	β_h-ep	10	4.9	45	2.8	183
2	Morphine	10	2.3	132	12.8	2400

Parameters are calculated from the data of Fig. 1.

Calculation of Pharmacokinetic Data. These data were calculated by using a semilogarithmic plot of the plasma levels of β_h-ep and a biexponential concentration time equation of the form $C = Ae^{-\alpha t} + Be^{-\beta t}$, in which C = plasma concentration at any time (14); A and B are the zero time intercepts; α and β are the disposition rate constants; and t equals time. The constants α and β, obtained by nonlinear regression analysis, can be used to calculate the distribution and elimination half-lives; $t_{1/2}\alpha = 0.693/\alpha$ and $t_{1/2}\beta = 0.693/\beta$. The plasma clearance (Cl) or metabolic clearance rate was estimated from X/AUC, in which X is the dose administered and AUC is the area under the plasma level versus time curve. The apparent volume of distribution (V_d) is equal to Cl/β. The disposition of β_h-ep in CSF was calculated by using a monoexponential equation (14), $t_{1/2} = 0.693/K$, in which K is the apparent first-order elimination constant, to obtain the half-life in CSF and the volume of distribution, V_d = dose/C_0, in which C_0 is the initial concentration obtained by extrapolation to time zero.

Source of Materials. β_h-ep was synthesized by the solid-phase method as described (15). The enkephalin analog was synthesized by the solid-phase method by Michael Rosenblatt of the Massachusetts General Hospital (Boston, MA). Morphine sulfate for clinical use was purchased from Eli Lilly.

Table 2. Hormonal responses to IV opioids

Drug	Dose, mg	Subject	Prolactin Basal, ng/ml	Prolactin Peak, ng/ml	Prolactin Peak time, min	Prolactin % increment	Growth hormone Basal, ng/ml	Growth hormone Peak, ng/ml	Thyrotropin Basal, μU/ml	Thyrotropin Peak, μU/ml
β_h-ep	1	Patient 1	21.6	32.0	5	48	0.6	3.3	1.8	1.6
	5	Patient 1	18.8	22.2	15	18	0.7	0.6	1.0	1.8
	10	Patient 2	3.8	10.2	30	168	0.3	0.5	2.1	1.5
	10	Patient 3	5.0	15.5	15	310	0.3	<0.3	<1.0	<1.0
Morphine sulfate	10	Patient 2	6.6	29.2	120	342	0.8	1.8	<0.6	2.5
	10	Patient 5	11.0	22.2	30	102	0.2	0.2	3.9	4.0
	10	Patient 6	8.0	22.8	30	185	5.4	1.4	2.2	2.7
[2-D-Alanine,5-norleucine]-enkephalinamide	2	Baboon 1	5.0	6.4	15	28	24	22.0*	0.4	0.5
	4	Baboon 2	6.2	10.6	15	71	<1.0	<1.0	<0.3	<0.3

* Baboon 1 had an elevated basal growth hormone level. Plasma concentration decreased to 6 ng/ml 60 min after the administration of the enkephalin analog.

RESULTS

After iv administration of 5 or 10 mg of β_h-ep, plasma levels of β_h-ep decreased rapidly at first and then more slowly (Fig. 1), so that the plasma concentration-time curve could be analyzed by the use of a biexponential equation. The resulting pharmacokinetic data are given in Table 1. The terminal half-life of β_h-ep ($t_{1/2}\beta$) averaged 37 min; the V_d averaged 178 ml/kg, and the Cl averaged 3.2 (ml/min)/kg. Pharmacokinetic data for morphine after administration to patient 2 (see Table 1) revealed that, compared to β_h-ep, the $t_{1/2}\beta$ for morphine was approximately 3 times longer; the V_d was 10-fold greater; and the Cl was 2½ times more rapid. No detectable increase in CSF β_h-ep concentrations occurred in patient 3 at any time during 24 hr after the injection of iv β_h-ep, although plasma samples showed increased β_h-ep concentrations. The administration of iv β_h-ep caused no significant change in pain, mood, pupil size, or vital signs. In contrast, significant increases in prolactin levels were detected as early as 5 minutes after β_h-ep administration. Data on basal and peak plasma hormone levels and the time of the peak prolactin level are summarized in Table 2. Increments in prolactin levels were greater after 10 mg of β_h-ep than after 1 or 5 mg iv. No significant increases in plasma growth hormone or thyrotropin levels were detected. CSF prolactin, growth hormone, and thyrotropin were measured simultaneously in patient 3; there was no significant change in the levels of these three hormones in the CSF. After 10 mg of iv morphine, plasma prolactin was increased from 102 to 342% (Table 2). Both morphine and β_h-ep caused a rapid rise of plasma prolactin levels with restoration of basal levels by 2 hr after β_h-ep and 4 hr after morphine (Fig. 2). In the baboon, no detectable objective behavioral effects or changes in vital signs were noted after 2 or 4 mg iv of the enkephalin analog. Plasma prolactin levels increased without any increase in growth hormone or thyrotropin levels. In fact, in baboon 1, whose basal growth hormone levels were elevated, a marked decrease in growth hormone occurred by 60 min (Table 2).

The icv administration of 7.5 mg of β_h-ep to patient 4 caused substantial changes in pain, mood, and pupil size. Such changes were not detected with lower icv doses from 0.1 to 5 mg. Analgesia and behavioral alterations began within 15 min after drug administration and the effects were maximal at 3 hr (Fig. 3). After 5 hr the patient was still drowsy, and scalp electroencephalograph recordings showed bilateral theta activity without focal or paroxysmal features. The patient did not request analgesic medication until 21 hr after β_h-ep administration, at which time all subjective and objective effects had disappeared.

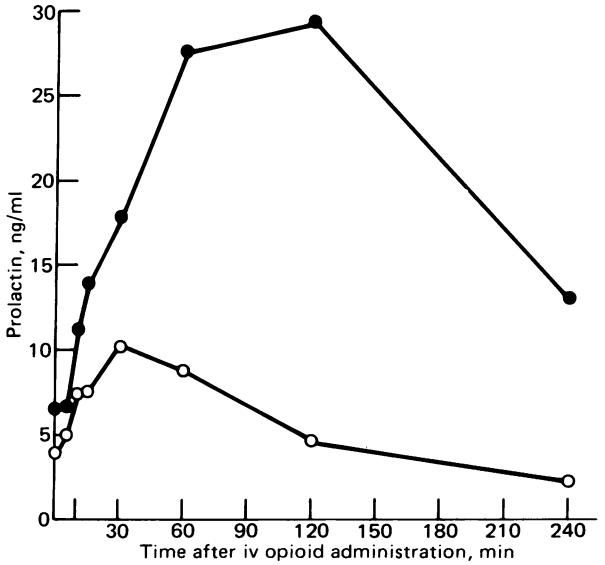

FIG. 2. Effect on plasma prolactin levels of 10 mg of β_h-ep (○) and 10 mg of morphine sulfate (●) iv given to patient 2 on separate occasions. Plasma growth hormone and thyrotropin levels did not increase after the administration of either opioid.

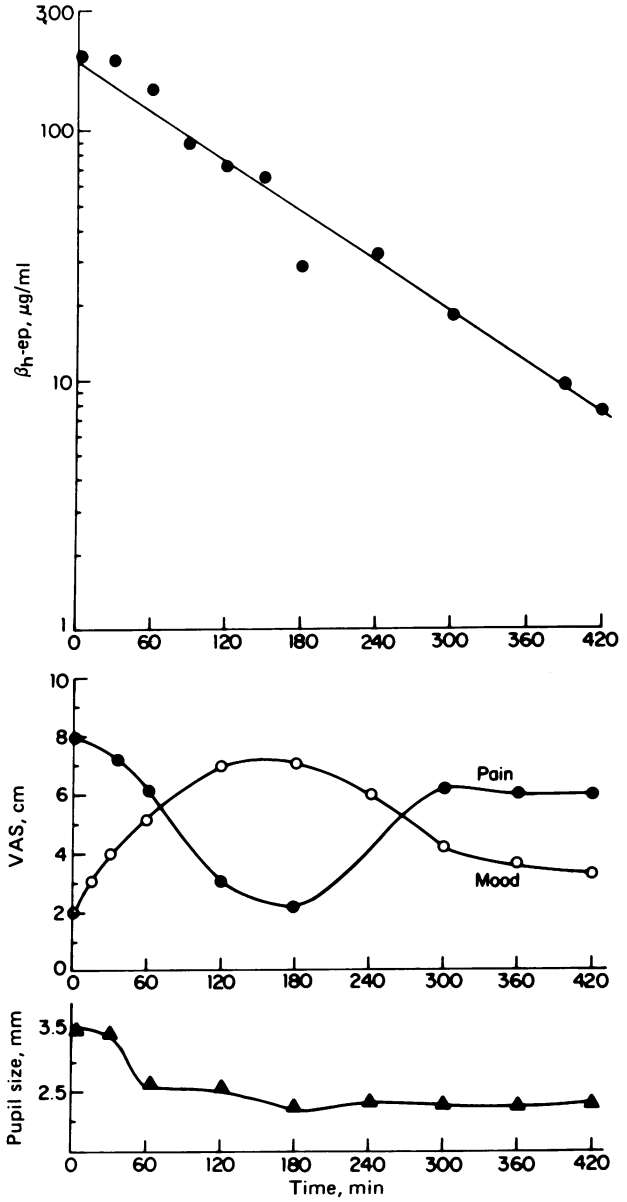

FIG. 3. Changes in CSF concentration of immunoreactive β_h-ep (●) after icv administration of 7.5 mg of β_h-ep and concurrent changes in pain (●), mood (○), and pupil size (diameter) (▲).

The disposition rate of β_h-ep from CSF after icv administration of 7.5 mg is shown in Fig. 3. The decline in CSF β_h-ep levels over time can be described by a monoexponential equation yielding a $t_{1/2}$ of 93 min and a V_d of 0.74 ml/kg. The levels of β_h-ep in CSF continued to decline for at least 21 hr. β_h-ep could not be detected in any of the concurrently collected plasma samples from 5 min to 21 hr. At 1 hr after drug administration, when a significant change had occurred in pain, mood, and pupil size, the ventricular CSF level was 150 μg/ml, and at 3 hr, when peak drug effects occurred, the CSF level was estimated at approximately 50 μg/ml (Fig. 3). The estimated

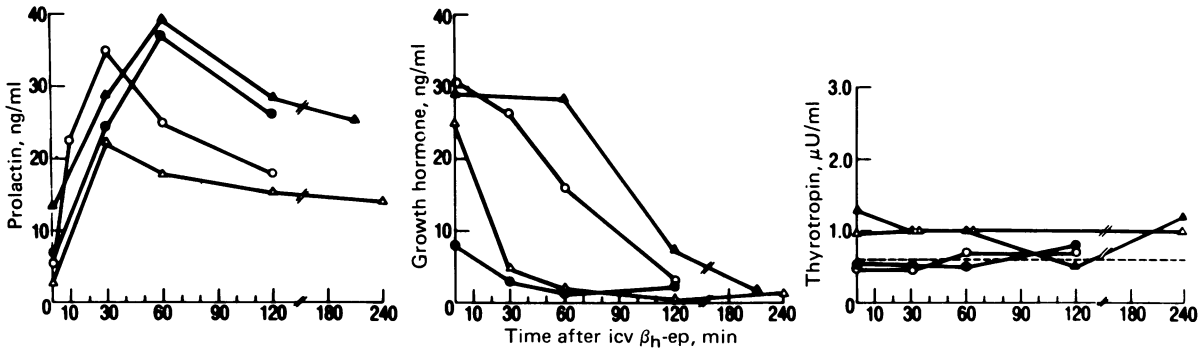

FIG. 4. Various doses of β_h-ep [0.1 mg (●), 0.2 mg (○), 1.6 mg (▲), 7.5 mg (△)] administered icv to patient 4 caused increases in plasma prolactin, decreases in plasma growth hormone, and no change in plasma thyrotropin. Changes in CSF prolactin were minimal and occurred 2 hr or more after β_h-ep administration, suggesting absorption from plasma. The broken line indicates the detection limit of the thyrotropin RIA.

V_d for β_h-ep given icv suggests that the drug is initially localized to the ventricular system. Although analgesic and behavioral changes occurred only after the administration of 7.5 mg of β_h-ep icv, hormonal effects were detected after 100, 200, and 1600 μg of β_h-ep icv (Fig. 4). Plasma prolactin levels rose within 10 min and remained elevated at 4 hr. Plasma growth hormone levels decreased after the administration of each of the four doses of β_h-ep. There was no significant change in plasma thyrotropin levels.

DISCUSSION

Exogenously administered β_h-ep is rapidly eliminated from the plasma. Its $t_{1/2}\beta$ (37 min) is similar to that reported by Liotta *et al.* (16) for exogenously administered adrenocorticotropin (33 min), a peptide of similar molecular weight; β-lipotropin had a $t_{1/2}\beta$ of 45 min. Calculating the other pharmacokinetic parameters from the data published by Liotta *et al.* (16) and comparing these data to our data for β_h-ep, we find that the Cl of β_h-ep and adrenocorticotropin is about half as rapid as that of β-lipotropin; the V_d of adrenocorticotropin (126 ml/kg) is smaller than that of β_h-ep, whereas the V_d of β-lipotropin (434 ml/kg) is larger than that of β_h-ep (16). Without knowing definitively whether there is any protein binding of these peptides, no firm conclusion can be drawn as to the basis of this difference in the V_d. However, all of these peptides are significantly more restricted in their distribution than morphine (Table 1).

In contrast, the $t_{1/2}$ of β_h-ep in CSF was significantly longer than in plasma, with immunological activity detectable for 21 hr. The slow disappearance of β_h-ep in CSF may account for the prolonged analgesic effects we have observed and the similar prolonged effects reported by others with brief electrical stimulation of the periventricular grey areas (17).

Although only icv injection of β_h-ep caused analgesia, neuroendocrine effects were observed after both iv and icv β_h-ep administration. The iv doses from 1 to 10 mg produced a rapid rise in prolactin. No detectable levels of β_h-ep could be measured in concurrently sampled human CSF, suggesting that β_h-ep penetrates the blood–brain barrier poorly if at all. Because β_h-ep does not stimulate prolactin release directly from pituitary cells in culture (18), it probably acts at the level of the median eminence, where there is no blood–brain barrier (19). The lack of analgesic and behavioral effects after iv injection relates to the inability of this agent to reach central sites in amounts requisite to produce such effects.

The icv administration of β_h-ep also produced increased plasma prolactin levels and decreased plasma growth hormone levels after doses ranging from 0.1 to 7.5 mg. These neuroendocrine effects occurred without behavioral and analgesic effects until 7.5 mg was given, with the neuroendocrine effects preceding the profound changes in mood, pain intensity, and pupil size. The time and dose differences between the neuroendocrine effects and analgesic and behavioral responses could be due to differences in proximity of receptors to the ventricular surface, differences in receptor affinity, or different receptors. The large doses required to produce analgesia in patient 4 may be related to the fact that he was chronically taking narcotic drugs. It is interesting to note that the neuroendocrine effects did not show the same degree of tolerance, again suggesting functional differences in receptor populations.

The neuroendocrine effects of opioid peptides appear to be species dependent. The rapid rise in plasma prolactin after iv β_h-ep without any increase in plasma growth hormone is similar to the effect of iv morphine in humans (20); this finding contrasts with the effect of β_h-ep in rats, in which stimulation of both growth hormone and prolactin secretion has been reported (21, 22). It has previously been reported that morphine stimulation of growth hormone secretion in the rat is a dose-related phenomenon and does not occur at doses of 50 μg/kg or less (23); similar dose-related effects in rats have been shown with β_h-ep (21, 22). Nevertheless, with the icv doses of β_h-ep that we have administered, there was decreased plasma growth hormone secretion in the presence of increased plasma prolactin. Furthermore, the administration of an enkephalin analog to another primate, the baboon, also produced increased plasma prolactin levels without an increase in growth hormone. In the one baboon with an elevated growth hormone level, plasma growth hormone actually decreased. These findings in two primate species demonstrate that opioid peptides may have different hormonal effects in primates than in nonprimates.

In severe pain or stress, conditions in which growth hormone may be elevated, β_h-ep appears to suppress the elevated growth hormone levels. Morphine has previously been observed to suppress the rise in growth hormone associated with major surgery (24). The elevated basal growth hormone level in patient 4 may have been related to his chronic use of narcotic analgesics (25) or may have been due to his chronic pain. After four different doses of icv β_h-ep administered to this patient, plasma growth hormone levels decreased. Only one report has documented both growth hormone and prolactin stimulation in humans after iv administration of an enkephalin analog (26). The difference between the results of these other workers and ours may be related to the analog used, its dose, or the fact that their patients were healthy volunteers rather than patients with chronic pain.

In conclusion, β_h-ep is rapidly cleared from human plasma,

but more slowly from CSF. The neuroendocrine effects observed appear to be species dependent and are similar to the effects of morphine in humans. β_h-ep stimulated prolactin release at doses significantly lower than those required to produce analgesic and behavioral effects. When both effects were observed after 7.5 mg icv, the onset of the hormonal and analgesic effects of β_h-ep may have resulted from the activation of opiate receptors that differ in their locations and characteristics.

We thank Dr. Michael Rosenblatt of the Massachusetts General Hospital for the synthesis of the enkephalin analog used in these studies. The various reagents for our RIAs were provided by the Hormone Distribution Program of the National Institute of Arthritis, Metabolism, and Digestive Diseases. We also thank Ada Rogers and George Heidrich for their expert nursing assistance. Expert technical assistance was provided by Beth Hoffman, Margaret Kelliher-Gibson, and Julia Liang. This research was supported in part by U.S. Public Health Service Grants DA-01707, CA-23185, CA-08748 and MH-30245, and by the Rita Allen Foundation.

1. Bloom, F., Siegal, D., Ling, N. & Guillemin, R. (1976) *Science* **194,** 630–632.
2. Loh, H. H., Tseng, L. F., Wei, E. & Li, C. H. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 2895–2898.
3. Guillemin, R., Vargo, T., Rossier, J., Minick, S., Ling, N., Rivier, C., Vale, W. & Bloom, R. (1977) *Science* **197,** 1367–1369.
4. Catlin, D. H., George, R. & Li, C. H. (1978) *Life Sci.* **23,** 2147–2154.
5. Hosobuchi, Y. & Li, C. H. (1978) *Commun. Psychopharmacol.* **2,** 33–36.
6. Catlin, D. H., Hui, K. K., Loh, H. H. & Li, C. H. (1978) in *Advances in Biochemical Psychopharmacology,* eds. Costa, E. & Trabucchi, M. (Raven, New York), pp. 341–350.
7. Foley, K. M., Inturrisi, C. E., Kourides, I. A., Kaiko, R. F., Posner, J. B., Houde, R. W. & Li, C. H. (1978) in *Characteristics and Function of Opioids,* eds. Van Ree, J. M. & Terenius, L. (Elsevier/North-Holland, Amsterdam), pp. 421–422.
8. Shapiro, W. R., Young, D. F. & Mehta, B. (1975) *N. Engl. J. Med.* **293,** 161–167.
9. Folstein, M. F. & Luria, R. (1973) *Psychol. Med.* **3,** 479–486.
10. Marquardt, W. G., Martin, W. R. & Jasinski, D. R. (1967) *Int. J. Addict.* **2,** 301–304.
11. Spector, S. (1971) *J. Pharmacol. Exp. Ther.* **178,** 253–258.
12. Kourides, I. A., Weintraub, B. D., Rosen, S. W., Ridgway, E. C., Kliman, B. & Maloof, F. (1976) *J. Clin. Endocrinol. Metab.* **43,** 97–106.
13. Bigos, S. T., Ridgway, E. C., Kourides, I. A. & Maloof, F. (1978) *J. Clin. Endocrinol. Metab.* **46,** 317–325.
14. Gibaldi, M. & Perrier, D. (1975) in *Drugs and the Pharmaceutical Sciences,* ed. Swarbrick, J. (Dekker, New York), Vol. 1, pp. 1–95.
15. Li, C. H., Yamashiro, D., Tseng, L. F. & Loh, H. H. (1977) *J. Med. Chem.* **20,** 325–328.
16. Liotta, A. S., Li, C. H., Schussler, G. C. & Krieger, D. T. (1978) *Life Sci.* **23,** 2323–2330.
17. Hosobuchi, Y., Rossier, J., Bloom, F. & Guillemin, R. (1979) *Science* **203,** 279–281.
18. Rivier, C., Vale, W., Ling, N., Brown, N. & Guillemin, R. (1977) *Endocrinology* **100,** 238–242.
19. Rapoport, S. I. (1976) *Blood-Brain Barrier* (Raven, New York), pp. 77–78.
20. Tolis, G., Hickey, J. & Guyda, H. (1975) *J. Clin. Endocrinol. Metab.* **41,** 797–801.
21. Chihara, K., Arimura, A., Coy, D. H. & Schally, A. (1978) *Endocrinology* **102,** 281–284.
22. Dupont, A., Cusan, L., Garon, M., Labrie, F. & Li, C. H. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 358–359.
23. Lien, E. L., Clark, D. E. & McGregor, W. H. (1978) *FEBS Lett.* **88,** 208–211.
24. George, T. M., Reier, C. E., Lanese, R. R. & Rosner, T. M. (1974) *J. Clin. Endocrinol. Metab.* **38,** 736–741.
25. Reed, J. L. & Ghodse, A. H. (1973) *Br. Med. J.* **2,** 582–585.
26. Stubbs, W. A., Jones, A., Edwards, C. R. W., Delitala, G., Jeffcoate, W. J., Ratter, S. J., Besser, G. M., Bloom, S. R. & Alberti, K. G. M. (1978) *Lancet* **ii,** 1225–1227.